at the single-molecule level. Our results suggest that G6pDH stochastically fluctuates between active—inactive states, favoring the active state upon closure of the tweezers. Our discovery may represent a general approach for refining nanodevices for advanced applications.

939-Plat
Regulation of Lipid Membrane Trafficking and Transmembrane Signaling by Graphene
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Cholesterol, a lipid molecule found ubiquitously in eukaryotic cells, plays a vital role in the integrity, dynamics, and trafficking of the lipid membrane, in addition to influencing many transmembrane proteins. However, the functionality of membrane cholesterol is far from clear, largely due to an inability to manipulate membrane cholesterol with high spatiotemporal precision. Popular tools like statins or methyl-β-cyclodextrin (MβCD) only lead to chronic and indiscriminative cholesterol reduction. Moreover, there is no selective approach to increase membrane cholesterol. Our recent work involving carbon nanomaterials provided an unexpected answer. Graphene, a one-atom thick carbon crystal, has been explored for biomedical applications because of its remarkable chemical and physical properties. Using in vitro and in vivo measurements, we have found that graphene selectively interacts with cholesterol. This enriches cholesterol at the plasma membrane, and thus enhances membrane lipid phase order, likely promoting the formation of cholesterol-rich lipid membrane nanodomains. Neurons grown on graphene exhibited presynaptic potentiation, specifically caused by a larger pool of releasable vesicles and an increase of fast recycling. By addition or depletion of membrane cholesterol, we found that the graphene-induced presynaptic enrichment of membrane cholesterol is necessary and sufficient to promote potentiation. In non-neuronal cells, graphene significantly elevates ATP-induced intracellular Ca^{2+}-signaling by promoting the activation of P2Y receptors, a group of GPCRs which are selectively responsive to extracellular ATP. Furthermore, we found that graphene enhances P2Y receptor signaling on the timescale of seconds, as rapidly as its effect on membrane packing. This then reveals an intriguing interaction between graphene and cholesterol, and its impact on plasma membrane structure, trafficking, and transmembrane proteins. Given the current challenges in manipulating membrane cholesterol, this graphene-cholesterol interaction will accelerate studies of membrane cholesterol function and broaden the biological application of carbon nanomaterials.

940-Plat
Development of a Fluorescence-Based Assay for Functional Studies of Transporter Proteins on the Single Molecule Level
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Regulated and selective transport of biomolecules across biological membranes is mediated by transporter proteins. These are essential in physiological processes ranging from electrical signaling in the nervous system to secretion of hormones and maintenance of electrochemical gradients. Though transporters are extensively studied, there are currently no techniques available to investigate their function at the single molecule level. We have developed a fluorescence-based assay to monitor thousands of individual nanoscale proteoliposomes in parallel by immobilizing them on functionalized glass surfaces (1-5).
We recently extended this assay to allow real-time observation of substrate translocation mediated by single transporters reconstituted into liposomes. Here we present the development of the assay including characterization and optimization of the most critical system components including: minimization of leakage, in situ real-time calibration of absolute transport rates and increase of photostability for long term recordings (~40min). We also present high-resolution single molecule activity recordings of the transporter protein, Arabidopsis thaliana H+-ATPase (AHA2).

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Symposium: Epigenetics

941-Symp
Cost and Precision in Small Gene Regulatory Networks
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Genes in early fly development are expressed with astonishing precision, despite the molecular noise intrinsic to all biochemical reactions. In an attempt to understand the regulatory mechanisms in this system, I will discuss the nature of hunchback mRNA expression based on analysis of live imaging experiments. Inspired by fly development, I will then discuss trade-offs that gene regulatory circuits have to face in order to precisely respond to signals in the presence of molecular noise.

942-Symp
Metaphase Chromatin Plates Explain the Structure and Physical Properties of Condensed Chromosomes
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Previous studies showed that during mitosis chromatin filaments are folded into multilayer plates (1). These structures can be self-assembled from chromatin fragments obtained by micrococcal nuclease digestion of metaphase chromosomes (2). Chromosomes of different animal and plant species show great differences in size (which are dependent on the amount of DNA that they contain), but in all cases chromosomes are elongated cylinders that have relatively similar shape proportions (the length to diameter ratio is approximately 13). It is possible to explain this morphology by considering that chromosomes are self-organizing supramolecular structures formed by stacked layers of planar chromatin having different nucleosome-nucleosome interaction energies in different regions (3). The nucleosomes in the periphery of the chromosome are less stabilized by the attractive interactions with other nucleosomes and this generates a surface potential that destabilizes the structure. Chromosomes are smooth cylinders because this morphology has a lower surface energy than structures having irregular surfaces. The symmetry breaking produced by the different values of the surface energies in the telomeres and in the lateral surface explains the elongated structure of the chromosomes. The results obtained by other authors in nanomechanical studies of chromatin and chromosome stretching have been used to test the proposed supramolecular structure. It is demonstrated quantitatively that internucleosome interactions between chromatin layers can justify the work required for elastic chromosome stretching. Chromosomes can be considered as hydrogels with a lamellar liquid crystal organization. The good mechanical properties of this structure may be useful for the maintenance of chromosome integrity during mitosis. Furthermore this chromatin organization avoids random entanglement of the extremely long genomic DNA molecules in chromosomes.
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943-Symp
Cooperativity and Supercoiling Modulate Functions of Human O^6-alkylguanine DNA Alkyltransferase
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Human cells contain DNA alkyltransferases that protect genomic integrity under normal conditions but also defend tumor cells against chemotherapeutic alkylating agents. Here we explore how structural features of the DNA substrate affect the binding and repair activities of the human O^6-alkylguanine-DNA alkyltransferase (AGT). In vitro, cooperative binding results in all-or-nothing association on short templates. A requirement for contact with 4 DNA base-pairs results in oscillation of average binding site size S_{app} and cooperativity factor w with template length. Models in which protein molecules overlap along the DNA contour predict that protein-protein contacts will be optimal when the DNA is torsionally relaxed. Supporting this prediction, topoisomerase assays show that AGT binding is accompanied by a